REMARKS

Claims 6, 9, 10, 12 to 15, 51, and 53 are pending in the subject application. Applicants have amended claims 6, 9, 10, 12, 13, 15, 51, and 53 herein.

Support for the amendments to claims 6 and 51 may be found, *inter alia*, in the specification as originally filed at page 2, lines 14 to 18; and page 8, lines 9 to 15.

Support for the amendments to claim 9 may be found, *inter alia*, in the specification as originally filed at page 7, lines 27 to 29.

Support for the amendments to claims 10 and 13 may be found, *inter alia*, in the specification as originally filed at page 7, lines 27 to 29; page 8, lines 9 to 15; and page 14, lines 19 to 27.

Support for the amendments to claim 12 may be found, *inter alia*, in the specification as originally filed at page 2, lines 14 to 18; and page 7, lines 27 to 29.

Support for the amendments to claim 15 may be found, *inter alia*, in the specification as originally filed at page 2, lines 14 to 18; and page 12, lines 8 to 12.

Applicants also have amended claim 53 herein to correct a typographical error. Accordingly, after entry of this amendment, claims 6, 9, 10, 12 to 15, 51, and 53 will be pending and under examination.

Claim Rejection Under 35 U.S.C. § 103(a) - Abstract of Strieter et al. in view of Le et al. PCT

The Examiner rejected claim 6 under 35 U.S.C. § 103(a) as allegedly unpatentable over the abstract of Strieter et al. (Strieter, R.M., et al. (1993) "Role of tumor necrosis factor-α in disease states and inflammation," Crit. Care Med. 21(10 Suppl.):S447-S463) in view of Le et al. PCT (PCT International Application No. WO 92/16553 A1, published October 1, 1992). The Examiner's specific rationale is set forth on page 2, line 13, to page 4, line 12, of the May 6, 2008 Final Office Action.

Applicants' Response

In response, without conceding the accuracy of the Examiner's position and in order to expedite prosecution, applicants have amended claim 6. Applicants maintain that the rejection cannot be applied to claim 6, as amended herein, for at least the reasons given below.

As an initial matter, applicants note that M.P.E.P. § 2141.02(VI) (8th Ed., 6th Rev., Sept. 2007) states that "[a] prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention" (emphasis in original). In accordance with M.P.E.P. § 2141.02(VI), applicants will refer to the entire disclosure of Streiter et al. herein, rather than to the abstract of Streiter et al. alone. Applicants attach hereto

as **Exhibit A** a copy of Streiter et al. for the Examiner's convenience.

1. There was no reasonable expectation of success of the combination of Streiter et al. with Le et al. PCT

Streiter et al. disclose results obtained from an *in vivo* model of hepatic ischemia-reperfusion injury in pathogen-free rats at page S453, left-hand column, line 27, to page S454, right-hand column, line 43. Le et al. PCT discloses that neither monoclonal antibody A2 nor chimeric antibody cA2 neutralizes or inhibits murine tumor necrosis factor-α (page 11, lines 14 and 15, and Figure 5). Furthermore, Le et al. PCT does not disclose neutralization of rat tumor necrosis factor-α by antibodies or fragments thereof.

Applicants maintain that Le et al. PCT does not disclose an anti-human tumor necrosis factor-α monoclonal antibody or fragment thereof that would be effective in the rat model of Strieter et al. Furthermore, applicants maintain that there was no reasonable expectation that the antibodies or fragments thereof of Le et al. PCT would be effective in the *rat* model of Strieter et al. because at least because neither monoclonal antibody A2 nor chimeric antibody cA2 neutralizes or inhibits *mouse* tumor necrosis factor-α. Therefore, applicants maintain that there was no reasonable expectation of success of the combination of Le et al. PCT with Streiter et al.

2. The Examiner incorrectly discounted Freeman and Nathanson's disclosure of unpredictability of the art

The Examiner discounted the disclosure of Freeman and Natanson because i) the studies used the Bay-X-1351 antibody; ii) none of the studies used the cA2 chimeric antibody; and iii) Le et al. PCT teaches that chimeric antibody cA2 is an improvement over the prior art at page 3, line 26, to page 4, line 12, of the May 6, 2008 Final Office Action.

Applicants respectfully maintain that the Examiner has not indicated why the use of the Bay-X-1351 antibodies by Freeman and Natanson, rather than the chimeric antibody cA2 of Le et al. PCT, is relevant to the discounting of Freeman and Natanson's disclosure. Furthermore, the Examiner has not indicated why the asserted improvements of chimeric antibody cA2 over the prior art is relevant to the discounting of Freeman and Natanson's disclosure. Additionally, applicants note that the Examiner asserted that "Freeman and Natanson are limited instances of failure to prolong survival" at page 4, line 10, of the May 6, 2008 Final Office Action. Applicants maintain that Freeman and Natanson disclose unpredictability in the art even if the disclosure of Freeman and Natanson is a "limited instance", which applicants do not concede.

In view of the foregoing, applicants maintain that claim 6, as amended herein, is not obvious over the abstract of Streiter et al. in view of Le et al. PCT Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claim Rejections Under 35 U.S.C. § 103(a) - Le et al. Patent in view of Bender et al.

The Examiner maintained the rejection of claims 6, 9, 10, 12 to 15, and 53 under 35 U.S.C. § 103(a) as allegedly unpatentable over Le et al. Patent (U.S. Patent No. 5,656,272) in view of Bender et al. (U.S. Patent No. 5,317,019). The Examiner's specific rationale is set forth on page 4, line 14, to page 5, line 13, of the May 6, 2008 Final Office Action.

Applicants' Response

In response, without conceding the accuracy of the Examiner's position and in order to expedite prosecution, applicants have amended claims 6, 9, 10, 12 to 15, and 53. Applicants maintain that the rejection cannot be applied to claims 6, 9, 10, 12 to 15, and 53, as amended herein, for at least the reasons given below.

Applicants maintain that there was no reasonable basis of success of the combination of Le et al. Patent and Bender et al. Specifically, the Examiner indicated a reliance on the alleged nexus between TNF and myocardial infarction or stroke on page 5, lines 7 to 8, of the May 6, 2008 Final Office Action. Bender et al. indicate at column 21, lines 20 to 25, that:

> TNF also has pro-inflammatory activities which together with its early production (during the intial [*sic*] stage of an inflammatory event) make it a likely mediator of tissue injury in several important disorders including but not limited to,

myocardial infarction, stroke and circulatory shock. (Emphasis added)

Applicants maintain that, at most, Bender et al. *suggest* that TNF is a "likely" mediator of "myocardial infarction, stroke and circulatory shock". Applicants further maintain that one of ordinary skill in the art would not conclude that Bender et al. discloses a nexus between tumor necrosis factor-α and myocardial infarction or stroke. Applicants therefore maintain that there was no reasonable basis of success of the combination of Le et al. Patent and Bender et al.

In view of the foregoing, applicants maintain that claims 6, 9, 10, 12 to 15, and 53, as amended herein, are not obvious over Le et al. Patent in view of Bender et al. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Claim Rejections Under 35 U.S.C. § 103(a) - Le et al. Patent in view of Bender et al. and in further view of Naughton et al.

The Examiner maintained the rejection of claims 6, 9, 10, 12 to 15, and 53 under 35 U.S.C. § 103(a) as allegedly unpatentable over Le et al. Patent and Bender et al. as applied to claims 6, 9, 10, 12 to 15 and 53, above, and in further view of Naughton et al. (U.S. Patent No. 5,863,531). The Examiner's specific rationale is set forth on page 5, line 15, to page 6, line 3, of the May 6, 2008 Final Office Action.

Applicants' Response

In response, without conceding the accuracy of the Examiner's position and in order to expedite prosecution, applicants have amended claims 6, 9, 10, 12 to 15, and 53. Applicants maintain that the rejection cannot be applied to claims 6, 9, 10, 12 to 15, and 53, as amended herein, for at least the reasons given below.

Applicant maintain that there was no reasonable expectation of success of the combination of Le et al. Patent, Bender et al., and Naughton et al. Specifically, Naughton et al. disclose at column 5, lines 2 to 13, that:

> For example, in the case of vascular grafts, the stromal cells can be genetically engineered to express anticoagulation gene products to reduce the risk of thromboembolism, atherosclerosis, occlusion, or anti-inflammatory gene products to reduce risk of failure. For example, the stromal cells can be genetically engineered to express tissue plasminogen activator (TPA), streptokinase or urokinase to reduce the risk of clotting. Alternatively, the stromal cells can be engineered to express anti-inflammatory gene products, e.g., peptides or polypeptides corresponding to the idiotype of neutralizing antibodies for tumor necrosis factor (TNF), interleukin-2 (IL-2), or other inflammatory cytokines" (emphasis added).

Applicants maintain that Naughton et al. discloses "tissue plasminogen activator (TPA), streptokinase, or urokinase" as examples of "anticoagulant gene products" (single underline), and discloses "[a]lternatively" "the idiotype of neutralizing antibodies for tumor necrosis factor (TNF)" as an example of

an "inflammatory cytokine" (double underline). Applicants further maintain that Naughton et al. does not disclose that "the idiotype of neutralizing antibodies for tumor necrosis factor (TNF)", or even a "anti-human tumor necrosis factor-α monoclonal antibody or antigen-binding fragment thereof" of claims 6, 9, 10, 12 to 15, and 53, as amended herein, can prevent thromboembolism. Applicants therefore maintain that one of ordinary skill in the art would have no reasonable basis of success for the combination of Le et al., Bender et al., and Naughton et al.

In view of the foregoing, applicants maintain that claims 6, 9, 10, 12 to 15, and 53, as amended herein, are not obvious over Le et al. Patent in view of Bender et al. and in further view of Naughton et al. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Previous Objections and Rejections Withdrawn

The Examiner acknowledged that all other rejections and objections as set forth or maintained in the prior office action are withdrawn in light of applicants' amendments.

Allowable Claim

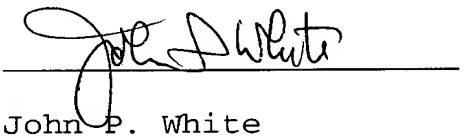
The Examiner acknowledged that claim 51 is free of the art.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants'

undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the enclosed $490.00 fee for a two-month extension of time, is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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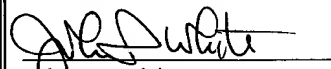
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EXHIBIT A

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Role of tumor necrosis factor-α in disease states and inflammation

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Objective: **To review the animal and human data defining the role of tumor necrosis factor (TNF) in the pathogenesis of the septic shock syndrome, the systemic inflammatory response syndrome, and related pathologic states.**

Data Sources: **The international English language literature from 1975 to present formed the basis for this review. MEDLINE was used to identify pertinent animal and human studies.**

Study Selection: **Those animal and human studies that focused on the mechanisms of action of TNF, its role in the inflammatory cytokine network, and the potential uses of anti-TNF therapies were emphasized.**

Data Extraction: **Animal studies were selected based on the relevance of the model to the pathogenesis of the human systemic inflammatory response syndrome. Where they provided supportive evidence, human studies were selected on the basis of study design.**

Data Synthesis: **TNF plays a major role in systemic inflammatory response syndrome secondary to infection, burns, trauma or hemorrhagic shock, and pancreatitis. TNF influences the outcome of other infectious processes, including allograft rejection, ischemia-reperfusion injury, delayed-type hypersensitivity, and granuloma development. The administration of anti-TNF antibodies, soluble TNF receptors, and related fusion proteins may limit organ damage and decrease mortality rate.**

Conclusions: **Although the regulated release of TNF may exert normal physiologic effects, the uncontrolled production of TNF may lead to organ dysfunction and death. TNF mediates a variety of other physiologic processes that are unrelated to sepsis syndrome. New anti-TNF therapies appear to attenuate the injurious actions of TNF. (Crit Care Med 1993; 21:S447–S463)**

KEY WORDS: tumor necrosis factor; inflammation; sepsis; organ failure, multiple; cytokines; critical illness; shock, septic

Tumor necrosis factor (TNF) has fascinated biomedical research scientists around the world and has received impressive attention during the last decade in several fields of biomedical research. This polypeptide mediator is a mononuclear phagocyte- and T-lymphocyte-derived cytokine that is being recognized with increasing frequency as an important agonist in a number of inflammatory and immunologically mediated responses (1–7). Although TNF was originally recognized for its oncolytic effects on solid tumors (8), subsequent investigations (1–7) demonstrated a pleiotropic effect in mediating both acute and chronic inflammatory/immunologic disorders. However, the scientific interest in studying the role of this cytokine *in vivo* is not restricted to molecular biologists or immunologists, but includes cellular biologists, physiologists, pharmacologists, and clinicians. It is doubtful whether there is any organ of the body that is not affected by TNF. This cytokine produces marked changes in the heart (9), lungs (10), central nervous system (11), liver (12), gut (13), kidneys (14), and skeletal system (15). As other articles in this supplement make clear, the more we learn about the actions of TNF, the more apparent it is that TNF is one of the body's primary mediators of inflammation, probably acting early in the sequence of events that produce inflammation and related conditions, such as sepsis and multiple organ dysfunction. The interest generated by these diverse scientific disciplines has provided the impetus for much of the investigative furor over TNF.

TNF has also been implicated in the pathogenesis of a wide variety of noninfectious disorders, including rheumatoid arthritis (16), systemic lupus erythematosus (17), coal workers' pneumoconiosis (18), alcoholic hepatitis (19), biliary cirrhosis (20), bowel necrosis (21), and multiple sclerosis (22, 23). In addition, increased

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circulating TNF concentrations have been found in patients with congestive heart failure (24, 25), and they are a marker of increased mortality rate in patients who initially survive cardiopulmonary resuscitation (26). It has even been suggested that increased TNF levels may predict early death in some elderly patients (27).

Interestingly, the biological importance of TNF correlates with the magnitude of its expression *in vivo* (Fig. 1). For example, at concentrations locally produced, TNF may be instrumental in the maintenance of physiologic homeostasis. At these tonically low levels, TNF may regulate a number of normal physiologic events, such as the circadian rhythm of body temperature, sleep, and appetite (6). As the concentrations of TNF increase in response to a local injury, this inflammatory mediator is believed to play a critical role as an early response cytokine in cellular activation and the propagation of the inflammatory response. The presence of TNF under these circumstances could orchestrate a localized inflammatory event and exert both autocrine and paracrine effects on the surrounding cells. In this context, the major role of either secreted or membrane-bound TNF would be to initiate, maintain, and resolve local inflammation. The importance of TNF and other cytokines in mediating local inflammation is often overlooked, as self-limiting inflammation is an extremely complex, yet understudied event.

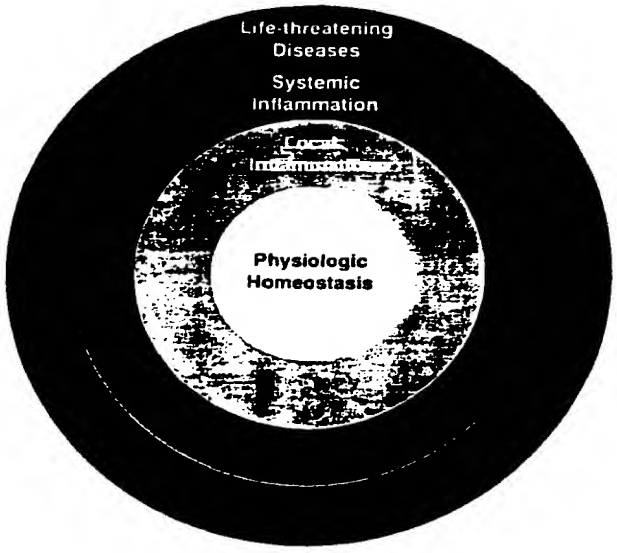

Figure 1. The biological effect of tumor necrosis factor correlates with the magnitude of its production.

The finding of significantly increased TNF concentrations *in vivo* during various disease states has generated interest from many biomedical research circles, as the increase appears to correlate with the pathophysiology of the disease (1–7). Several cell-mediated, chronic inflammatory responses are mediated via a cascade of cytokine releases that are important in promoting the cellular interactions that culminate in an immune response. Often, these cytokines reach high enough concentrations to escape the local inflammatory region and may then be found in the systemic circulation. The presence of circulating cytokines systemically causes immune cell activation and a significant alteration of the host's physiology. The exaggerated production of TNF under these conditions may result in pathophysiologic events and the generation of symptoms associated with disease. Alterations of body temperature resulting in fever, loss of appetite, cachexia, and lethargy are all TNF-induced changes that were once thought to be a normal physiologic response (6). Finally, the uncontrolled and overexuberant production of TNF has been associated with high morbidity and mortality rates. Perhaps the most studied example of this life-threatening situation is the role of TNF in mediating the pathophysiology of sepsis and multiple organ failure. This devastating event is an example of cytokine dysregulation. Such dysregulation often fails to respond to conventional intervention and may lead to the host's death.

It is clear that, in many patients with what we have been calling "sepsis," no site of infection can ever be documented. A new phrase, the "systemic inflammatory response syndrome," has been coined to describe this disorder (28). Strong evidence suggests that TNF plays a central role in the development of the systemic inflammatory response syndrome, regardless of whether it is of infectious or noninfectious origin.

Thus, the importance of TNF as a key, early-response mediator in inflammation appears to be associated with the magnitude of its expression *in vivo*. In this manner, TNF can exert diverse effects on a number of both acute and chronic disease states. These biological effects reflect the broad range of cytokine activities: TNF has numerous pleiotropic effects on a number of cellular functions, demonstrates a biphasic dose-response, is regulated in a complex fashion, and can influence cellular systems via a cascade of events. In this review, information is provided that assesses the role of TNF in disease states other than sepsis and multiple organ failure, and explores the potential mechanism whereby TNF can both initiate and maintain the inflammatory response that is associated with these diseases.

TUMOR NECROSIS FACTOR-INDUCED CYTOKINE NETWORKS

Increasing scientific evidence supports the hypothesis that an inflammatory response is initiated, maintained, and finally resolved via the interplay of cytokine networks. The subsequent biological response is characterized by the capacity of an early response cytokine, synthesized by one cell, to induce the production of different cytokines in an autocrine, paracrine, or hormonal manner. The ability of TNF to orchestrate different events necessary for the successful initiation, maintenance, and resolution of inflammation is exemplified by the diverse array of cytokines whose release is induced by TNF. For example, TNF can cause the expression of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemotactic protein-1, as well as its own gene in a number of immune and nonimmune cells (2, 4, 6, 29–32).

The role of TNF as an early response cytokine through the mediation of cytokine networks in the lung is schematically shown in Figure 2. In this paradigm, the early expression of TNF plays a central role in the induction of cytokine networks essential to monocyte recruitment to an area of inflammation within the lung (32). In this situation, resident macrophages (both alveolar and interstitial macrophages) rapidly respond to an inciting agent or immune challenge with the elaboration of TNF. In turn, this initial production of TNF activates multiple events in a paracrine manner, leading to the expression of adhesion molecules on the surface of endothelial cells and the production of monocyte chemotactic protein-1 by nonimmune cells. This complex event culminates in the successful recruitment of monocytes to a specific area of inflammation. The mechanism outlined for the initiation of inflammation and leukocyte recruitment is not restricted to the lung, but is most likely a series of events that can occur in all tissues. The importance of TNF in directing cellular communication during an inflammatory response is related to the presence of TNF receptors on all somatic cells, which allows their participation in inflammation.

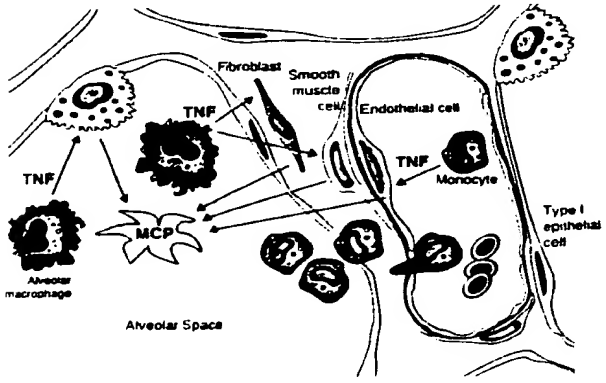

Figure 2. Tumor necrosis factor (*TNF*)-dependent cytokine networks within the lung that elicit the infiltration of monocytes. *MCP*, monocyte chemotactic protein.

ROLE OF TUMOR NECROSIS FACTOR IN IMMUNE REACTIVITY

The role of TNF in the mixed lymphocyte reaction is an excellent example of this cytokine's ability to mediate a cascade of events essential to the subsequent sensitization of lymphocytes and resulting in the proliferative mixed lymphocyte reaction. The mixed lymphocyte reaction has previously been used to analyze cytokine activation pathways and T-lymphocyte proliferation. In addition, this system has been regarded as a model that simulates the immunologic responses that are associated with allograft rejection. TNF plays an intricate role in the mixed lymphocyte reaction, as it can be identified as one of the earliest cytokines produced after the initiation of this reaction (33, 34). The pattern of TNF expression is biphasic: The earliest detectable levels of TNF are found within the first few hours of the mixed lymphocyte reaction, followed by a decline over the next 2 to 4 days, and finally with a precipitous rise at days 5 and 6. This latter increase in TNF levels during the mixed lymphocyte reaction is coincident with a maximal proliferation of T-lymphocytes. Moreover, the neutralization of TNF by specific antibodies during the mixed lymphocyte reaction results in a significant attenuation of lymphocyte proliferation. A potential mechanism on which the early expression of TNF in the mixed lymphocyte reaction appears to be dependent upon leukocyte adhesion molecule interactions. The neutralization of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, or lymphocyte functional antigen-3, all of which are involved in the mixed lymphocyte reaction, can inhibit T-lymphocyte sensitization and subsequent proliferation (35–42). However, reconstitution with exogenous TNF in the mixed lymphocyte reaction reestablishes the lymphocyte proliferative response (34). Furthermore, the influence of TNF on the induction of cytokine networks in the mixed lymphocyte reaction is also exemplified by its ability to produce a number of chemotactic cytokines. This latter event may be important in attracting mononuclear cells to the site of an antigen/alloantigen immune response. Thus, it would appear that TNF plays a central role as the mediator of T-lymphocyte sensitization in the response to an antigenic challenge.

The above data support the notion that TNF is an important cytokine, mediating a number of inflammatory and immune events.

The diverse effects of TNF in physiologic, immunologic, and pathologic systems have provided the impetus to fully determine its role in a number of inflammatory and immune-mediated disorders. Studies have both corroborated our understanding of TNF's role in mediating infectious disease pathophysiology and have shown that TNF plays a greater role in noninfectious immune disease state pathogenesis than had previously been thought. For example, TNF appears to be a key cytokine in both the response to parasitic and viral diseases, and in the pathogenesis of graft vs. host disease, allograft rejection, and ischemia-reperfusion injury. These latter observations are especially important, as they demonstrated the participation of TNF in pathologic disorders that are unrelated to infection.

ROLE OF TUMOR NECROSIS FACTOR IN TRANSPLANTATION AND ALLOGRAFT REJECTION

Recently, several studies demonstrated that TNF is intimately involved in immunoregulation. These investigations showed that TNF can affect B-lymphocyte differentiation and enhance the generation of cytotoxic T lymphocytes (43–45). Additionally, high-affinity TNF receptors have been found on the surface of activated (nonresting) T lymphocytes and are expressed in a temporal manner similar to that of interleukin-2 (IL-2) receptors (46). These T-lymphocyte-derived, high-affinity receptors for TNF possess important biological function, as lymphocytes exposed to TNF display an augmented expression of major histocampatibility complex class II antigens and high-affinity IL-2 receptors. In addition, stimulation with TNF is synergistic with IL-2 stimulation, leading to accentuated T-lymphocyte sensitization and proliferation, as well as the production of γ-interferon. Furthermore, TNF has been shown to stimulate T-lymphocyte colony formation, an event that is potentially mediated through IL-1 production and enhanced antigen-induced T-lymphocyte proliferation (47, 48). These studies stress the importance of TNF as an early response protein that is necessary for induction of the cytokine cascades that promote an immune response. TNF in the presence of alloantigen has been an important mediator in the evolution of a mixed lymphocyte reaction and the generation of cytotoxic T lymphocytes.

Perhaps the most important evidence that TNF is involved in the pathogenesis of transplant/allograft rejection stems from both clinical and experimental investigations. In clinical studies, increased concentrations of TNF were found in association with renal allograft rejection (49). Additionally, high concentrations were coincident with clinical and histopathologic evidence of orthotopic liver allograft rejection (50). Interestingly, increased TNF levels were found 1 to 2 days preceding clinical evidence of liver allograft rejection, while they were further increased in association with clinical and histopathologic evidence of allograft rejection. Similarly, increased TNF levels were found immediately after kidney reimplantation, as well as 1 to 2 days before the pathologic confirmation of renal allograft rejection. Thus, increased concentrations of TNF have now been documented in association with the pathogenesis of human liver, kidney, and heart allograft rejection.

The direct role of TNF in mediating allograft rejection has been shown using animal models of allograft rejection. Animals that were passively immunized with TNF-neutralizing antibodies provided interesting findings about allograft survival. For example, passive immunization with neutralizing antibodies to TNF can prolong the survival of skin allografts in Rhesus monkeys (51). Similar results were obtained from experiments using animal models of heterotopic heart transplantation. In this model, the donor heart (brown Norway rat) is transplanted to the cervical location of a recipient Lewis rat. In unmodified control animals, cardiac rejection usually occurs on day 7 after reimplantation. Histopathologically, the heart allograft rejection is characterized by a significant mononuclear cellular infiltrate that surrounds the myocardial cells, an observation that is compatible with the classical cell-mediated immune response. Immunohistochemical analysis for the presence of an *in situ* antigenic expression of TNF demonstrated a specific staining pattern for this cytokine that was primarily associated with mononuclear cell infiltrates (52). These results were similar to the immunohistochemical staining pattern of TNF in heart allografts of humans who had died because of rejection.

These findings support the notion that specific therapeutic agents that either target the suppression of TNF synthesis or block the biological activity of TNF may prove useful in attenuating cardiac allograft rejection. To assess the potential role of "anti-TNF therapy," other investigations tested the timing of TNF-neutralizing antibody treatment. A single dose of the agent administered on the day of the transplant proved to have significant therapeutic effect and was highly efficacious in delaying the onset of heart allograft rejection, extending it to 13 days, compared with 7 days for rats that did not receive the anti-TNF antibody. This finding was comparable with allograft recipients who

had received either dexamethasone or cyclosporine (52). Interestingly, delaying the administration of neutralizing antibodies to TNF for 24 and 72 hrs after reimplantation proved to be equally effective. The mean heart allograft survival in these two groups was 16 and 11 days after reimplantation, respectively. However, if the neutralizing antibodies to TNF were administered 5 days after reimplantation, no significant difference in allograft survival was found. Furthermore, the exogenous administration of recombinant TNF resulted in the acceleration of heart allograft rejection. These findings are compelling, as they demonstrate a potential for the development of novel and specific therapies to prevent allograft rejection. The development of innovative agents that may reverse allograft rejection would be an exciting addition to the current therapeutic options available to treat transplant rejection.

To address the potential efficacy of combined immunotherapies, studies have been conducted to determine what the effect of combining cyclosporine with TNF-neutralizing antibodies would be with the cardiac allograft survival paradigm (53). Actuarial allograft survival demonstrated that passive neutralization of TNF in combination with low-dose (1.5 mg/kg/day) cyclosporine resulted in significantly increased allograft survival time, as compared with the effect of either agent alone. The administration of both anti-TNF antibodies and low-dose cyclosporine at either the time of transplantation or on day 3 or day 5 after reimplantation prolonged allograft survival threefold, as compared with the unmodified control group. Allograft survival in rats treated with both agents was two times longer than in groups treated with either neutralizing TNF antibodies or cyclosporine alone. Immunohistochemical localization of TNF from cardiac allografts of animals receiving the combined therapy demonstrated a significantly reduced pattern of staining for antigenic TNF. In addition, both the infiltration of cytotoxic T lymphocytes within the allograft and circulating levels of TNF were dramatically reduced. While the exact mechanism for the synergistic effects of these two modes of immunosuppression is not entirely clear, it may occur because TNF inactivation and cyclosporine act at different levels of the developing immune response. For example, the neutralization of TNF may decrease lymphocyte activation and proliferation and may block the release of other cytokines as well. These mechanisms are consistent with TNF's ability to modulate the cytokine networks involved in the successful development of a mixed lymphocyte reaction. The role of cyclosporine in prolonging allograft survival may depend on its ability to attenuate the expression of interleukin-2. The reduction in IL-2 during cardiac transplantation would result in altered immune reactivity, including a decline in lymphocyte and monocyte reactivity via reduced cytokine production.

Lung transplantation recently became a therapeutic option for a number of end-stage pulmonary diseases (54). Although the popularity of lung transplantation increased dramatically over the last several years, the recipients of these allografts have had far greater acute and chronic transplantation-related complications than recipients of other solid organs (54–62). Virtually all lung allografts develop varying degrees of rejection within the first 4 wks after reimplantation (54, 62–64). In addition, chronic allograft rejection (obliterative bronchiolitis) is a major mechanism by which the loss of lung allografts occurs (56, 62, 65). This form of allograft rejection is an insidious complication that is usually related to chronic airway inflammation as a result of recurrent episodes of both infection and acute rejection (64, 65). Recent clinical evidence suggests that the aggressive measures to monitor and treat episodes of acute lung allograft rejection have led to a significant reduction in obliterative bronchiolitis. However, it is becoming clear that the pathologic mechanisms which result in lung allograft rejection are complex and new insight into the mechanisms of allograft rejection and novel therapies to abort transplant rejection will be needed to prolong lung allograft survival.

Recent investigations have begun to lay the foundation in this arena, as animal models of lung transplantation have become more successful. In particular, scientific advances have been made regarding the role of cytokines in lung allograft survival. Using a rat model of an unmodified histo-incompatible, specific pathogen-free, orthotopic lung transplantation, as compared with syngeneic isografts, investigations implicated TNF in mediating lung allograft rejection (66). It was found that TNF exerts a potential multifunctional role in mediating allograft injury; the cytokine was expressed early and in a compartmentalized fashion after reimplantation. In both lung allografts and isografts, peak TNF bioactivity was seen at 24 hrs after reimplantation. These findings are compatible with the increased concentrations of TNF that are seen in association with the ischemia-reperfusion injury that accompanies reimplantation. The magnitude of an initial ischemia-reperfusion injury and the generation of TNF within the allograft may play an important role in the development of a specific immune response and allograft rejection. Subsequent to the effects of ischemia-reperfusion, the lung injury subsides and only minor histopathologic alterations can be observed over the first 4 days after reimplantation. During this time, both steady-state concentrations of TNF mRNA and TNF bioactivity declined to concentrations equivalent to

those values of sham-operated control animals. However, in conjunction with the fulminant, acute allograft rejection seen during days 5 to 6 after reimplantation, the concentrations of TNF within the lung allografts were significantly increased, as compared with either the contralateral native lung or the isograft lungs. To determine whether the expression of TNF during the evolution of lung allograft rejection contributed to the pathogenesis of allograft rejection, animals were passively immunized with neutralizing antibodies to TNF on the day of reimplantation. Neutralization of TNF significantly attenuated lung allograft rejection on day 6 after reimplantation, as compared with control allografts.

The sequential phases of lung allograft rejection (Fig. 3) are probably initiated and maintained via the early production of mononuclear cell-derived TNF and the expression of additional cytokines and adhesion molecules. The initial reimplantation response, or latent phase, is associated with a nonspecific inflammatory reaction that is mechanistically analogous to the TNF-induced tissue injury that is seen in hepatic ischemia-reperfusion. During the early phases of lung allograft rejection, mononuclear phagocytes are the most important cellular source of TNF. This early production of TNF, subsequent to reimplantation, will have pleiotropic effects on the development of subsequent immune responses, including the expression of intercellular adhesion molecule-1 by endothelial cells within the allograft (Fig. 3*A*). In recipients of syngeneic isografts, similar patterns of TNF and intercellular adhesion molecule-1 expression can be seen; however, the expression of these molecules returns to concentrations equivalent to those values of sham-operated controls. In contrast, the immune response that occurs in the presence of alloantigen, which is mediated by TNF and intercellular adhesion molecule-1 expression, primes the allograft for the final destructive phase of rejection. During the vascular phase of lung allograft rejection (Fig. 3*B*), the expression of endothelial cell-derived intercellular adhesion molecule-1 targets the recipient's leukocytes within the allograft. This action is followed by the movement of the recipient's leukocytes into the allograft, which results in the development of perivascular and peribronchial mononuclear cell sequestration or bronchus-associated lymphoid tissue. Recruitment of the recipient's leukocytes into the bronchus-associated lymphoid tissue leads to a donor-recipient alloantigen interaction and the development of an "*in situ* mixed lymphocyte reaction." While TNF-mediated lymphocyte activation and proliferation are occurring within the lung allograft, a similar response is concurrently evolving systemically in the recipient's reticuloendothelial system. This phenomenon

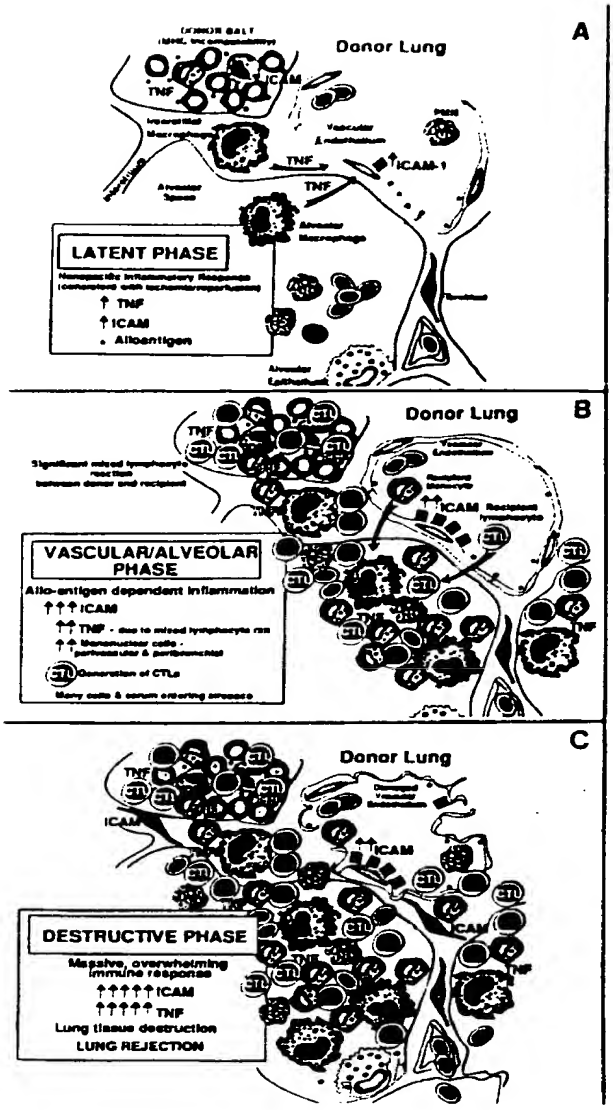

Figure 3. Role of tumor necrosis factor in mediating acute lung allograft rejection. *TNF*, tumor necrosis factor; *ICAM*, intercellular adhesion molecule; *BALT*, bronchus-associated lymphoid tissue; *CTL*, cytotoxic T lymphocytes; *MHC*, major histocompatibility complex.

culminates in the production of recipient cytotoxic T-lymphocytes, which circulate to the microvasculature of the lung allograft and mediate fulminant allograft rejection (Fig. 3*C*). The above findings demonstrate that TNF expression is bimodal in character during the full development of lung allograft rejection. The first peak in lung (transplant) TNF concentrations

correlates with ischemia-reperfusion injury or the reimplantation response and the second peak is associated with the development of a cell-mediated immune response culminating in allograft rejection.

The apparent pleiotropic role of TNF in organ rejection and the clinical availability of neutralizing TNF antibodies make this cytokine an appealing target for specific immunotherapy. This approach may be an efficacious strategy, especially when used in combination with cyclosporine. The ability to reduce the dosage of cyclosporine in these patients may have a profound effect on attenuating its toxic side-effects. Thus, availability of agents that will target the specific cytokines involved in cellular activation, such as TNF, may provide the clinician with novel therapeutic options in the near future. Other pharmaceutical agents that may alter the production or activity of TNF include prostaglandins of the E series, pentoxifylline, and amiloride (67–69). These agents may hold promise for the treatment of allograft rejection. The ability of different immunosuppressive agents to act via separate, distinct cellular or molecular mechanisms may eventually be the optimal combination immunotherapy for successful treatment of acute or chronic allograft rejection.

ROLE OF TUMOR NECROSIS FACTOR IN ISCHEMIA-REPERFUSION INJURY

The role of TNF in mediating ischemia-reperfusion injury has been referred to above in association with the reimplantation response. Additional studies further substantiated the role of TNF in mediating ischemia-reperfusion injury, particularly in the *in vivo* model of rat hepatic ischemia-reperfusion injury (70). These experiments were performed on pathogen-free rats. Ischemia of the liver's cephalad three lobes was induced for 90 mins, which avoided total hepatic ischemia and passive mesenteric venous hypertension. After this maneuver, liver enzyme (SGPT) concentrations were significantly increased over control (sham) operated animals. Moreover, microscopic examination of the liver 24 hrs after reperfusion demonstrated severe injury in the lobes that had been ischemic. This pattern of injury consisted of central vein hepatocellular necrosis and neutrophilic infiltration (Fig. 4*A*). While lipopolysaccharide could not be detected throughout the reperfusion period, a significant increase in TNF bioactivity was observed in the systemic circulation through the use of a cytolytic bioassay. Plasma TNF concentrations reached maximum (70 pg/mL) between 30 mins and 3 hrs after reperfusion, while the sham-operated control group concentrations of TNF were undetectable. Specificity for the biological activity of TNF was confirmed by adding TNF-neutralizing antibodies to the bioassay. Although the cellular source of TNF production and the mechanism by which it took place were not shown in these studies, the hepatic Kupffer cell was the most likely cellular source of this cytokine. Kupffer cells are the largest fixed-tissue macrophage population in the body and, collectively, they represent the most important potential cellular source for TNF. Thus, the temporal pattern of TNF expression in hepatic ischemia-reperfusion is consistent

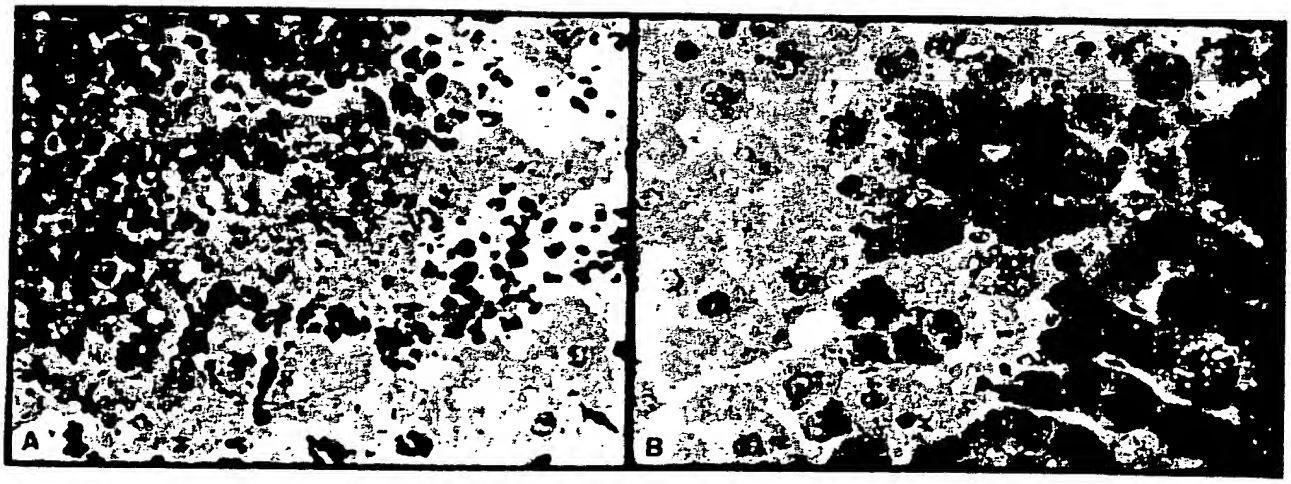

Figure 4. Toluidine blue staining of liver tissue postischemia-reperfusion injury. *A*) Liver injury associated with ischemia-reperfusion. Note the significant neutrophilic infiltration. *B*) Liver tissue posthepatic ischemia-reperfusion. Animals were passively immunized with neutralizing tumor necrosis factor antibodies before ischemia.

with the kinetics for the production of TNF from other models of organ ischemia-reperfusion injury.

Interestingly, not only did hepatic ischemia-reperfusion result in direct liver injury, but, as an indirect consequence, there was an induction of clinically important pulmonary pathologic alterations. Hepatic ischemia-reperfusion injury resulted in substantial accumulations of neutrophils within the pulmonary microvasculature and increased permeability of the alveolar-capillary membrane. The sequestration of neutrophils within the lung was measured by their myeloperoxidase activity. This activity was significantly increased in the lungs of animals that had undergone hepatic ischemia-reperfusion, as compared with sham-operated animals. These findings suggest that soluble mediators were released from the liver into the systemic circulation after ischemia-reperfusion injury and that these mediators had an effect on the lung microvasculature. The histopathology of the lungs confirmed an injury pattern that was consistent with a pulmonary microvascular injury characterized by edema, hemorrhage, and a neutrophilic alveolitis. To ascertain whether TNF played a central role in the development of hepatic ischemia-reperfusion-induced pulmonary injury, animals were passively immunized with TNF-neutralizing antibodies before hepatic ischemia. Neutralization of TNF in this paradigm resulted in a significant attenuation of hepatocellular transaminase levels, permeability of the lung microvasculature, lung myeloperoxidase activity, and neutrophil sequestration within the ischemic lobe of the liver after reperfusion (Fig. 4*B*). These findings support the notion that TNF that is released after the reperfusion of an ischemic liver also acts to attract leukocytes into organs distal to the injury site. Since TNF itself is not directly chemotactic for leukocytes, the mechanism for TNF-induced neutrophil extravasation must depend on the induction of additional inflammatory mediators, such as chemotactic cytokines and adhesion molecules. These findings suggest that TNF is an important molecule in these events and should be targeted, possibly by immunotherapy, to reduce ischemia-reperfusion injury of an organ.

ROLE OF TUMOR NECROSIS FACTOR IN DELAYED-TYPE HYPERSENSITIVITY AND GRANULOMA DEVELOPMENT

The clinical manifestations of delayed-type hypersensitivity granulomatous inflammation are usually the consequence of an intense immune and nonimmune cellular interaction, and are often accompanied by tissue destruction followed by end-stage fibrosis. These inflammatory states may be difficult to treat clinically, and at times they may require therapy that compromises immune system function. Clinically, the strategy to treat delayed-type hypersensitivity granulomas depends on whether or not there is an infectious etiology. The treatment of granulomas caused by infectious organisms is to identify the pathogens and treat them with specific antimicrobial agents. In contrast, the clinical management of granulomas that are not infectious in nature involves targeting the patient's immune cell function with immunosuppressive agents. However, successful diagnosis and subsequent clinical treatment of the various delayed-type hypersensitivity diseases not necessarily restrict the progression of their pathology. This fact is exemplified in drug-resistant mycobacterial or fungal diseases or in progressive refractory sarcoidosis. The limited number of therapeutic options available to effectively manage these patients may reflect our limited knowledge of the mechanisms that underlie these chronic delayed-type hypersensitivity granulomatous diseases.

The initiation and maintenance of delayed-type hypersensitivity responses are a result of a dynamic interaction between the inciting agent and the host's immune and nonimmune cells. Histologically, the progression of delayed-type hypersensitivity responses is characterized by a mixed cellular infiltrate composed of newly recruited monocytes, macrophages, lymphocytes, and other cellular populations in various states of activation (epithelioid cells, endothelial cells, giant cells, and fibroblasts). Although many of the delayed-type hypersensitivity reactions possess a stereotypic histology, the intensity and chronicity of the response may vary dramatically. The intensity or immune reactivity of the developing granulomatous response allows us to define granulomas as either a delayed-type (cell-mediated) or foreign body-type granulomatous response. This classification is based on the degree of antigen-specific sensitization associated with the developing lesion. The delayed-type hypersensitivity lesions are antigen-driven responses with an active and sustained leukocyte recruitment, while the foreign body-type response lacks both an antigen-specific component and sustained leukocyte elicitation.

While both of the above granulomatous lesions have been well characterized at the histologic level, many of the molecular and cellular events necessary to initiate, maintain, and resolve these reactions have not yet been fully elucidated. Presumably, the observed pathophysiology of these diseases is due to the increased concentrations of inflammatory mediators within the evolving lesions. The generation of cytokines and the expression of adhesion molecules are responsible for the biology of the delayed-type hypersensitivity response. Specific cytokines have been isolated and identified

from delayed-type hypersensitivity lesions and include interleukins, TNF, interferons, fibroblast growth factors, and colony-stimulating factors (7). These findings provide circumstantial evidence for the role of cytokines in the delayed-type hypersensitivity response. However, they have not provided mechanistic information regarding the contribution of specific cytokines to the development of the lesion.

Recent investigations provided new insight into the role of TNF in these responses and demonstrated a causal relationship between TNF and the initiation and maintenance of cell-mediated delayed-type hypersensitivity responses. This cytokine is especially important in orchestrating leukocyte recruitment and activation, as well as in committing resident, nonimmune cells to participate in the developing lesion. A number of animal model studies support this concept of the role of TNF in the maintenance of the delayed-type hypersensitivity granulomatous response. In a murine model of mycobacterial-induced Bacille bilié de Calmette-Guérin granulomatous disease, the development of delayed-type hypersensitivity granulomas was causally related to the local production of TNF (71).

In this particular study, four interesting observations were made regarding TNF production and the delayed-type hypersensitivity response. First, there was a direct correlation between the expression of TNF mRNA, the concentrations of TNF, and the evolution and eventual regression of the Calmette-Guérin bacillus-induced delayed-type hypersensitivity lesions. Second, the direct effects of TNF appeared to occur locally, since systemic TNF concentrations were not detectable during the various phases of delayed-type hypersensitivity development. Thus, TNF appears to be compartmentalized to the microenvironment of the granuloma. Third, passive immunization with neutralizing TNF antibodies reduced both the size and the number of granulomatous lesions. Moreover, the most important finding of this study was that neutralization of TNF appeared to be a useful therapeutic option, as fully developed Calmette-Guérin bacillus-induced granulomatous lesions were shown to regress after passive immunization. This latter point underscores the essential fact that the presence of TNF is required for the persistence of a well-developed granuloma. Finally, TNF itself was found to be important in the induction of mononuclear cell-derived TNF, as neutralization of TNF resulted in the cessation of TNF synthesis. These investigators speculated that the early production of TNF in these lesions was released from activated lymphocytes. This notion is supported by the fact that T lymphocytes, when activated via the combined CD-3 and CD-28 pathways, are rich cellular sources of TNF (72). However, as the delayed-type hypersensitivity response evolves to a more cellular phase-like phenomenon, mononuclear phagocytes become the predominant cellular source of TNF in the microenvironment of the granulomatous lesion. The release of TNF within the local delayed-type hypersensitivity granulomatous lesion is an amplifying signal and, in the presence of antigen, perpetuates the immune response.

Other compelling evidence for the involvement of TNF in delayed-type hypersensitivity granuloma formation can be demonstrated using a model of synchronously developing *Schistosoma mansoni* egg-induced lung granulomas (7). In this model of the delayed-type hypersensitivity response, TNF concentrations are relatively low during the initiation phase, but increase significantly during the maintenance phase of the evolving granuloma. These findings are in contrast to the temporal expression of IL-1, which peaks early during the evolution of the delayed-type hypersensitivity response and is followed by a decline to unmeasurable concentrations during the maintenance phase of the evolving granulomatous lesion. These data support the notion that IL-1 is critical to the early recruitment and local cellular activation of the delayed-type hypersensitivity response, while TNF contributes to the continued organization of the maturing granuloma. Interestingly, foreign body-type pulmonary granulomatous lesions produce lower concentrations of IL-1 and TNF than do delayed-type hypersensitivity lesions. These observations reflect the degree to which sensitized T-lymphocytes participate in the developing granuloma. In additional studies using this model of synchronously developing *S. mansoni* egg-induced lung granulomas, the specific lymphocyte-derived mediators interleukin-4 (IL-4) and γ-interferon were found to influence the development of the immune reaction. The sequential production of IL-4 and γ-interferon was found to be temporally related to changes in granuloma macrophage production of TNF and superoxide anion. High concentrations of IL-4 were produced early in the initiation phase of delayed-type hypersensitivity granuloma formation in previously sensitized animals, whereas maximal γ-interferon production was found during the maintenance phase of the lesion. The maximal TNF concentrations correlated with both superoxide anion release and γ-interferon production. Passive immunization with IL-4-neutralizing antibodies resulted in a significant attenuation of the size of the developing lesion, while similar studies with γ-interferon-neutralizing antibodies had little effect. An additional piece of evidence supporting this idea of the role of TNF in the development of *S. mansoni* egg-induced granulomas was the finding that an administration of exogenous TNF to severe combined immunodeficiency

mice could reconstitute the generation of a granulomatous response (73).

SYSTEMIC INFLAMMATORY RESPONSE SYNDROME

The role of TNF in the development and maintenance of the systemic inflammatory response syndrome has also been a topic of much discussion. However, this discussion is hampered by difficulties and misconceptions about the disease entity itself. Diagnostic criteria for the systemic inflammatory response syndrome and related disorders were developed in a 1991 consensus conference (28) (Table 1). The conference was called because there was a clear lack of agreement in the medical community about the diagnostic criteria for sepsis (74). As a consequence, the patient populations in many studies of sepsis varied considerably (75), and it was difficult to compare the results of these studies in any meaningful way.

One of the challenges faced by the conference was that our understanding of what was then called "sepsis" had outpaced the words we used to describe it. For example, it was clear that many patients with sepsis never developed shock, yet they died a few weeks later of organ dysfunction (75); thus, the term "septic shock" was inadequate for describing the most severe stages of this disorder. Bacteremia occurs in less than half of all patients with sepsis and, in a sizable minority (at least 15%), no source of infection can ever be found (75). Furthermore, a similar (if not identical) disorder is known to result from noninfectious causes such as

Table 1. New definitions for systemic inflammatory response syndrome and related disorders

Infection	A microbial phenomenon characterized by an inflammatory response to the presence of microorganisms or the invasion of normally sterile host tissue by those organisms.
Bacteremia	The presence of viable bacteria in the blood. (The presence of other organisms in the blood should be described in a similar manner, i.e., viremia, fungemia, etc.)
Systemic inflammatory response syndrome (SIRS)	The systemic inflammatory response to a variety of severe clinical insults, including infection, pancreatitis, ischemia, multiple trauma and tissue injury, hemorrhagic shock, immune-mediated organ injury, and exogenous administration of inflammatory mediators such as tumor necrosis factor or other cytokines. SIRS is manifested by (but not limited to) two or more of the following conditions: Temperature: >38° C or <36 C°. Heart rate: >90 beats/min. Respiratory rate: >20 breaths/min or Pa_{o_2} <32 torr (<4.3 kPa). White blood cell count: >12,000 cells/mm^3, <4000 cells/mm^3, or >10% immature (band) forms. These changes should represent an acute alteration from baseline in the absence of another known cause for the abnormalities.
Sepsis	The systemic response to infection. This response is identical to SIRS, except that it must result from infection.
Severe sepsis	Sepsis associated with organ dysfunction, perfusion abnormalities, or hypotension. Perfusion abnormalities may include (but are not limited to) lactic acidosis, oliguria, and an acute alteration in mental status. Hypotension is defined as a systolic blood pressure <90 mm Hg or a reduction of >40 mm Hg from baseline in the absence of another known cause for hypotension.
Septic shock	Sepsis with hypotension (as defined above) despite adequate fluid resuscitation, in conjunction with perfusion abnormalities (as defined above). Patients who are on inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured, yet may still be considered to have septic shock.
Multiple organ dysfunction syndrome	Presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention. Primary multiple organ dysfunction syndrome is the direct result of a well-defined insult in which organ dysfunction occurs early and can be directly attributable to the insult itself. Secondary multiple organ dysfunction syndrome develops as a consequence of a host response and is identified within the context of SIRS.

These definitions were developed at a consensus conference jointly sponsored by the American College of Chest Physicians and the Society of Critical Care Medicine and held in August 1991 (28).

severe burns, trauma, hemorrhagic shock, pancreatitis, and the exogenous administration of cytokines (28).

A common pathway, i.e., widespread endothelial inflammation resulting in increased vascular permeability, is believed to underlie both sepsis and these other related disorders (76). Thus, the phrase "systemic inflammatory response syndrome" was coined to describe the clinical manifestations of this endothelial inflammation (28). (The word "sepsis" should be used only for patients with systemic inflammatory response syndrome in whom infection can be documented. In other words, all patients with sepsis have systemic inflammatory response syndrome, but not all patients with systemic inflammatory response syndrome have sepsis.)

If the endothelial inflammation is allowed to progress, considerable damage to a number of organ systems will ensue. Left unchecked, this damage will eventually lead to outright organ failure. The term "multiple organ dysfunction syndrome" was invented to describe the continuum from early dysfunction through organ failure (28).

What role does TNF play in the pathogenesis of endothelial inflammation, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome? The evidence for its role in patients with sepsis has been presented elsewhere, and will not be repeated here. Instead, we will focus on three of the most common noninfectious causes of systemic inflammatory response syndrome: burn injury, trauma/hemorrhagic shock, and pancreatitis. However, before evidence for the role of TNF in noninfectious causes of the systemic inflammatory response syndrome is presented, two caveats must be raised: a) Many studies of patients with sepsis, including several studies that assayed TNF concentrations, did not require documentation of infection as part of their definition of sepsis (77–83). Thus, a number of the patients included in these studies would meet the present definition of systemic inflammatory response syndrome, but not the criteria for sepsis. For example, in a study by Casey et al. (77), a documented site of infection could be demonstrated in only 44 of 97 patients diagnosed as having sepsis. Of the remaining patients, 38 had a clinical diagnosis of pneumonia, ten had peritonitis, two were thought to have urosepsis, one had culture-negative endocarditis, one had cellulitis, and one had pulmonary infarction. b) The interpretation of TNF concentrations measured in humans is hampered by several problems. First, it is unclear what constitutes a "normal" circulating TNF concentration. In a number of studies (78, 84, 85), healthy volunteers were found to have measurable TNF concentrations in their blood; in two reports (79, 84), these concentrations averaged roughly 75 pg/mL. Thus, early reports of "measurable" or "elevated" TNF concentrations must be interpreted with caution. Second, the normal release of TNF into the circulation of patients with the systemic inflammatory response syndrome seems to be phasic, and the half-life of this cytokine is short (86). Furthermore, there are two active forms of TNF (87) and the measurement of serum concentrations assays only one of them. Consequently, intermittent measurements of circulating TNF concentrations may be misleading.

Burns. Circulating TNF concentrations are significantly increased (mean, 264 pg/mL) within 2 days of a severe (second- or third-degree) burn (88). At this time, TNF concentrations correlate positively with body temperature and negatively with the white blood cell count. Thereafter, circulating TNF concentrations fluctuate, although the peaks do not seem to correspond with severity of illness (88).

Whether increased circulating TNF concentrations portend a poor prognosis in burn victims is unclear. In the study by Cannon et al. (88), mean TNF concentrations were higher in the patients who died than in the patients who survived, but the difference did not reach significance. In an earlier study by Marano et al. (89), patients who died of their burn injuries had significantly higher circulating TNF than did those patients who survived. Furthermore, the patients who died were more likely to have detectable TNF concentrations than were the patients who survived (71% vs. 31%, respectively).

Burn injury can prompt TNF release in at least two ways. Destruction of the cutaneous barrier allows microbes to invade the surrounding tissues and makes access to the bloodstream easier. Also, the burn injury itself releases tissue fragments and other factors that can prompt monocytes and macrophages to produce TNF (88, 90). Animal studies by Ogle et al. (91) showed that bone marrow macrophage TNF production is markedly enhanced for at least 1 day after burn injury. Prostaglandin E_2 production is also greatly increased, but this arachidonate metabolite seems unable to downregulate TNF release in this setting.

Ogle et al. (91) also showed that bone marrow macrophages from burned animals have increased cytotoxicity. They suggest that this finding may result from an increase in membrane-bound, or cell-associated TNF. Support for this hypothesis comes from Keogh et al. (87), who showed that murine liver and peritoneal cells produce two types of TNF, a 17-kilodalton circulating protein and a 29-kilodalton cell-associated protein. Cells obtained from rats that had been subjected to burn injury and infection produced markedly higher concentrations of both forms of TNF than did cells from healthy rats; however, the cell-associated protein was

synthesized more consistently than was the circulating form.

Such locally acting TNF could exert considerable effects, even in the absence of circulating cytokine concentrations. In the liver, cell-associated TNF could induce an acute-phase response, thereby decreasing albumin synthesis and upregulating other reactants (92). In bone marrow, cell-associated TNF could theoretically change the growth hormone milieu and, thus, alter the types of monocytes produced (93).

An obvious problem in assessing the role of burn injury in the development of the systemic inflammatory response syndrome is that it can be difficult to separate the effects of the burn injury itself from the effects of microbial invasion. In rats, it has been shown that both burned animals and burned/infected animals are more likely to have detectable circulating TNF concentrations than are healthy animals, but the mean TNF concentrations are much higher in the burn/infected rats than in the burned rats (94). In humans, however, we cannot always distinguish between patients who are merely severely burned, those patients who are burned and in whom bacterial products have translocated across the gut barrier, and those patients who are burned and actively infected. One study has suggested that the concentrations of TNF secreted by monocytes (both circulating and cell-associated forms) may be increased from 1 to 3 days before the onset of sepsis in burn victims and trauma patients (95), but this finding awaits confirmation.

Trauma/Hemorrhagic Shock. There is less direct evidence for the role of TNF in the development of the systemic inflammatory response syndrome among patients with trauma and/or hemorrhagic shock than there is for burn victims. Nevertheless, several lines of evidence strongly implicate this cytokine.

Trauma patients, like burn victims, have been shown to have an increased proportion of an unusual type of monocyte (the FcRI$^+$ monocyte) that has been linked to aberrant monocyte and lymphocyte function and to metabolic derangements (93). These monocytes, which express a 72-kilodalton receptor for immunoglobulin (Ig)G, produce elevated concentrations of cell-associated TNF, prostaglandin E_2, interleukins 1 and 6, and transforming growth factor-β (93). The increase in cell-associated TNF concentrations is particularly marked 3 days postinjury, and decreases thereafter (although it remains elevated through at least day 6).

Miller-Graziano et al. (93), suggested that the initial posttrauma trigger(s) for altered cytokine production can vary from patient to patient, and may include bacterial products as well as noninfectious agents such as substance P or complement split products. The altered mediator environment would then prompt the disproportionate development of FcRI$^+$ monocytes during myeloid differentiation. The discovery of these monocytes also provides a partial explanation for how the inflammatory response can get out of control. The interleukin-6 produced by these cells stimulates lymphocytes to release IgG; IgG, in turn, stimulates the FcRI$^+$ monocytes to even greater production of TNF and other mediators. The hormonal milieu is thereby altered even further, increasing the likelihood that more FcRI$^+$ cells will be produced.

The discovery of FcRI$^+$ monocytes may also help explain why TNF production is not downregulated by increased prostaglandin concentrations in patients with systemic inflammatory response syndrome: the FcRI$^+$ monocytes are insensitive to prostaglandin E_2 (93).

Furthermore, the fact that most of the TNF that is synthesized by these monocytes is cell associated rather than secreted, may help to explain why circulating TNF concentrations correspond poorly to clinical outcome in patients with a systemic inflammatory response syndrome resulting from many different underlying causes. In one study of patients with multiple accidental injuries, for example, there was no correlation between the circulating concentrations of TNF and the circulating concentrations of acute-phase proteins (96). However, tissue concentrations of cell-associated TNF were not assayed in this study. It is tempting to speculate, therefore, that we have been measuring the wrong form of TNF.

A study of patients with the adult respiratory distress syndrome (ARDS) lends support to this suggestion. Suter et al. (97) measured concentrations of TNF and other inflammatory mediators in both the plasma and bronchoalveolar lavage fluid of these patients, all of whom had either trauma, sepsis, or prolonged shock as the underlying cause of ARDS. Concentrations of TNF in the bronchoalveolar fluid were markedly increased during all stages of ARDS; mean values peaked during the early severe stage at 10,731 pg/mL. In contrast, plasma TNF concentrations remained within normal ranges throughout the stages of ARDS. These authors suggested that the TNF detected in bronchoalveolar lavage fluid is a cell-associated form, produced by pulmonary macrophages.

It should be noted, however, that other studies have found increased circulating TNF concentrations in patients with ARDS resulting from sepsis (or other infection), trauma, burns, or pancreatitis (98). The reasons for this discrepancy are unclear, but may relate to the phasic nature of circulatory TNF release.

TNF may also contribute to the onset of other forms of organ dysfunction after trauma and/or hemorrhagic shock. For example, in mice subjected to hemorrhage, hepatic Kupffer cells express significantly increased

concentrations of TNF and other cytokines for at least the first 24 hrs after the injury (99). Similarly, splenic macrophages taken from rats subjected to multiple hemorrhagic episodes produce markedly increased concentrations of TNF 48 hrs after injury (100). In this study, the duration of hemorrhage had more of an impact on dysregulation of immune function than did the extent of blood loss.

In a study of patients with severe blunt multiple trauma, head trauma, or septic shock, it was found that the patients with multiple trauma, like the patients with septic shock, had high concentrations of platelet sequestration in the lungs, liver, and intestines (101). Sequestration concentrations were increased in both survivors and nonsurvivors in these groups, but the concentrations in nonsurvivors were considerably more increased than were the concentrations in survivors. All six of the patients who died had multiple organ failure, as well as increased platelet sequestration. In contrast, the patients with head trauma had little or no platelet sequestration in the lungs, liver, or intestines. One of the primary causes of platelet sequestration is the local release of platelet-activating factor (102, 103); production of that mediator is prompted by TNF (21). Tumor necrosis factor also disrupts the balance between procoagulant and anticoagulant forces (104). (Obviously, platelet sequestration is not the only way in which TNF contributes to the onset of organ dysfunction; neutrophil recruitment and activation is another important mechanism. However, a complete review of the role of TNF in producing multiple organ dysfunction syndrome is beyond the scope of this article.)

Trauma, like burn injury, is frequently complicated by infection and, thus, the effects of TNF induced by trauma can be difficult to distinguish from the effects of TNF that are induced by infectious organisms. More research is needed to separate these issues.

Pancreatitis. One of the strongest arguments for the existence of a common inflammatory mechanism underlying acute pancreatitis and severe sepsis is the striking similarity of the clinical features associated with the two disorders, including the potential progression to multiple organ dysfunction syndrome (105). Evidence suggests that TNF may be one of the chief mediators of inflammation in patients with acute pancreatitis (106).

In one study (107), measurable concentrations of circulating TNF were found in 43% of the serum samples obtained from 38 patients with severe pancreatitis. Furthermore, higher initial TNF concentrations correlated with more severe outcomes. Animal studies (106) confirmed that TNF concentrations increase during the course of cerulein-induced acute pancreatitis. Furthermore, the increase in TNF concentrations is mirrored by the extent of edema formation in the lungs and pancreases of these animals. Surprisingly, when these animals were pretreated with an anti-TNF antibody, edema formation was enhanced, rather than blocked. The reason for this counterintuitive finding remains unclear.

Other animal studies (108) showed that the administration of TNF and interferon-γ induces piloerection, abdominal swelling due to ascites, thymic atrophy, splenic enlargement, and edematous enlargement of the pancreas. Histologic examination of these animals demonstrates a generalized infiltration of lymphocytes and polymorphonuclear cells in the pancreas, areas of hemorrhagic necrosis, ductal dilation, and intralobular and subcapsular edema. The islets are intact, however. A combination of TNF and interferon-γ also induces the expression of major histocompatibility complex-class I and class II molecules on ductal and acinar cells (108).

This discussion of the role of TNF in the pathogenesis of the systemic inflammatory response syndrome leads to a number of important conclusions for clinical practice.

a) The definition of systemic inflammatory response syndrome is highly nonspecific (28). A number of clinically unimportant processes can produce manifestations that would meet the definition for systemic inflammatory response syndrome. Thus, to more accurately pinpoint those patients who would previously have been defined as having sepsis and would therefore be considered at high risk for death, physicians should stratify patients with the systemic inflammatory response syndrome, using one of the scoring systems for illness severity. As new agents for the treatment of sepsis/systemic inflammatory response syndrome undergo clinical trials and become available for use, they should be administered only to patients who are found to have a high risk of death. (Trials of these agents will probably withhold them from patients assessed to be at extremely high [perhaps >90%] risk of death; whether these drugs can ethically be withheld from such patients in clinical practice remains to be determined.)

b) When a patient has signs and symptoms consistent with the definitions of systemic inflammatory response syndrome, it cannot be assumed that an infection is present. However, if the patient seems severely or even moderately ill, a thorough search for a possible site of infection is mandatory. If an infection is found (or strongly suspected), appropriate antibiotics should be administered. Surgical drainage should also be considered.

c) If a site of infection is found or strongly suspected, antibiotic selection should be considered carefully. It

cannot be assumed that the cause of the infection is a Gram-negative organism. The occurrence of Gram-positive sepsis has increased dramatically in recent years; in many recent studies, Gram-positive organisms were found to be a more common source of sepsis than were Gram-negative organisms (109). The occurrence of fungal sepsis has also sharply increased (110). The best approach to antibiotic selection may be to combine information from the patient history, knowledge of prevalence patterns for nosocomial infections at our own institutions, and recognition that sepsis may result from any of a number of organisms.

d) If no site of infection is found or strongly suspected, it is debatable whether antibiotics should be given. The decision may depend on the clinical setting; for example, it is probably wise to administer antibiotics to a burn patient (using the guidelines set forth above). It is well known that infection significantly increases the mortality rate in burn victims; thus, the potential advantages of antibiotic administration far outweigh the risks. In contrast, antibiotic administration may not be justified in patients with pancreatitis.

e) The difficulties (just described) in determining how and when to administer antibiotics highlight why new treatments, such as monoclonal antibodies to TNF, are urgently needed. Such treatments could potentially be given regardless of whether an infection is present and regardless of what type of organism may be causing the infection (111). These new treatments must be studied carefully, however. Although increased TNF concentrations have been shown to have many deleterious sequelae, we cannot overlook the possibility that this cytokine is also a key agent in the body's host defense system. Thus, any agent administered to counteract TNF has, theoretically, at least, the potential to do more harm than good.

ENDOGENOUS INHIBITORS OF TNF AND POTENTIAL FUTURE THERAPEUTIC INTERVENTIONS

While the above studies demonstrate that TNF plays a prominent role in the development of many immune-related responses, other studies have substantiated the importance of a regulated TNF response by identifying endogenous inhibitors of TNF. These host-derived, TNF-specific proteins block the biological activity of TNF (112). The inhibitory mechanism appears to work by interfering with the ability of TNF to form a functional ligand-receptor complex, thus preventing TNF-dependent signal transduction. The roles of TNF in immune-mediated events have taken on added significance with the identification of two host-derived, "shed" soluble TNF receptors or binding proteins. TNF has two distinct cell surface receptors designated as p60 and p80 that are classified according to their molecular weights of 60 kilodaltons and 80 kilodaltons, respectively (113). Recent evidence (114) showed that murine TNF receptors can mediate distinct cellular responses. Most soluble cytokine-binding proteins are the product of proteolytically cleaved, membrane-bound receptors. However, they may also be coded for by alternatively spliced mRNA coding (114). These soluble, extracellular, ligand-binding segments of specific cytokine receptors occur naturally and, therefore, have a potential role as endogenous regulators of cytokine bioactivity. The importance of soluble cytokine receptors in the regulation of inflammation is exemplified by the observation that various pox viruses encode proteins with both functional and structural homology to the extracellular portions of the TNF receptor, suggesting that these proteins aid the virus in the subversion of the host's immune response (115). Recently, a synthetic, dimeric, soluble fusion protein construct has been generated by combining two p60 TNF receptors with the Fc portion of a human IgG1 molecule (116). This fusion protein construct possesses a higher affinity and is a superior inhibitor of TNF bioactivity than either the native monomeric TNF receptor or anti-TNF antibodies. The use of novel therapies, such as endogenous cytokine inhibitors or neutralizing TNF antibodies to treat chronic inflammatory disease is now appreciated and should open an exciting era for the treatment of these enigmatic diseases.

CONCLUSIONS

TNF is a pleiotropic cytokine that is involved in mediating a number of cellular and molecular events essential to the full development of an inflammatory/immune response. While a number of eloquent studies demonstrated that TNF is an essential, proximal mediator of sepsis syndrome, the role of TNF in mediating inflammatory disorders that are unrelated to infectious etiologies is increasingly recognized. These latter diseases are often refractory to treatment with conventional therapy. The finding that these disorders can be attenuated through the use of TNF-neutralizing antibodies is exciting, and may open a new arena for the use of novel therapeutic strategies in the treatment of these diseases.

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